WETStar

User's Guide

The user's guide is an evolving document. Please check our website periodically for updates. If you find sections that are unclear, or missing information, please let us know.

WET Labs, Inc.
PO Box 518
Philomath, OR 97370
(541) 929-5650
www.wetlabs.com

WETStar Warranty

Standard Warranty

This unit is guaranteed against defects in materials and workmanship for two years from the original date of purchase. Warranty is void if the factory determines the unit was subjected to abuse or neglect beyond the normal wear and tear of field deployment, or in the event the pressure housing has been opened by the customer.

To return the instrument, contact WET Labs for a Return Merchandise Authorization (RMA) and ship in the original container. WET Labs is not responsible for damage to instruments during the return shipment to the factory. WET Labs will supply all replacement parts and labor and pay for return via 3rd day air shipping in honoring this warranty.

Annual Servicing Extended Warranty

WET Labs will extend the warranty on this unit to five years if it is returned annually for servicing. This includes calibration, standard maintenance, and cleaning. Charges associated with this annual service work and shipping are the responsibility of the customer.

Shipping Requirements for Warranty and Out-of-warranty Instruments

1. Please retain the original shipping material. We design the shipping container to meet stringent shipping and insurance requirements, and to keep your meter functional.

2. To avoid additional repackaging charges, use the original box (or WET Labs-approved container) with its custom-cut packing foam and anti-static bag to return the instrument.
   - If using alternative container, use at least 2 in. of foam (NOT bubble wrap or Styrofoam "peanuts") to fully surround the instrument.
   - Minimum repacking charge for WETStars: $25.00.

3. Clearly mark the RMA number on the outside of your shipping container and on all packing lists.

4. Return instruments using 3rd day air shipping or better: do **not** ship via ground.

Attention!

Return Policy for Instruments with Anti-fouling Treatment

WET Labs cannot accept instruments for servicing or repair that are treated with anti-fouling compound(s). This includes but is not limited to tri-butyl tin (TBT), marine anti-fouling paint, ablative coatings, etc.

Please ensure any anti-fouling treatment has been removed prior to returning instruments to WET Labs for service or repair.

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1. Specifications

Mechanical

	Pressure housing—6.7 x 2.7 in (17.1 x 6.9 cm)
Size:	Overall height (including bulkhead connector and tubing fittings)—10.2 in (25.7 cm)
Weight	in air: 1.7 lb (0.8 kg); in water: 0.25 lb (0.1 kg)
Rated depth:	600 meters
Housing:	Acetal copolymer

Electrical

Response time:	0.17 sec (analog); 0.125 sec (digital, optional)
Input:	7–15 VDC
Output:	0–5 VDC (analog); 0–4095 counts (digital, optional)
Current draw:	< 40 mA (analog); < 80 mA (digital, optional)
Linearity:	≥ 99% R^2

Optical

Chlorophyll

Dynamic ranges:	0.03 –75 µg/l (standard); 0.06–150 µg/l (optional)
Sensitivity:	≥ 0.03 µg/l
Excitation:	460 nm
Emission:	695 nm

CDOM

Dynamic ranges:	1000 ppb (estuarine waters) 250 ppb (near-coastal waters) 100 ppb (open ocean waters)
Sensitivity:	0.100 ppb quinine sulfate dihydrate (other sensitivities available on request)
Excitation:	370 nm (10 nm FWHM)
Emission:	460 nm (120 nm FWHM)

Uranine

Dynamic range:	0–4000 µg/l uranine
Sensitivity:	1 µg/l uranine
Excitation:	485 nm
Emission:	532 nm

Rhodamine

Excitation:	470 nm
Emission:	590 nm

Phycoerythrin

Excitation:	525 nm
Emission:	575 nm

Specifications are subject to change without notice.

1.1 Connectors

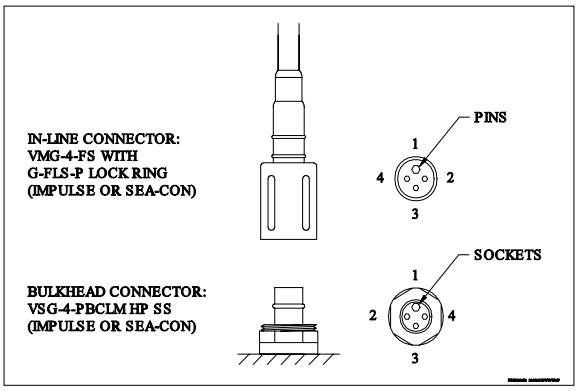

Figure 1. WETStar bulkhead connector

Pinout summary for four-pin WETStar connectors

Analog		Digital	
Socket/Pin	**Function**	**Socket/Pin**	**Function**
1	Common (ground)	1	Ground
2	Analog out	2	Analog +
3	V+	3	V+
4	Analog return	4	RS-232 (TX)

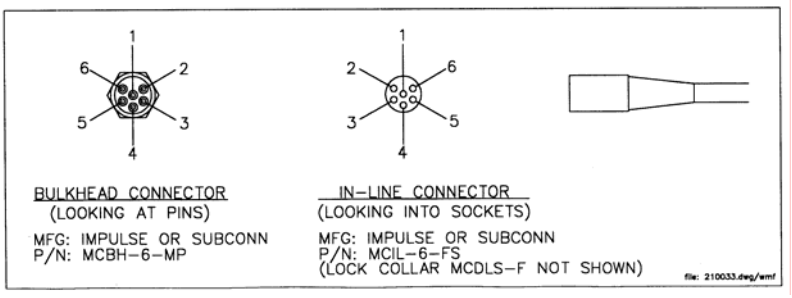

Figure 2. Optional 6-pin digital WETStar connector

Pinout summary for optional digital WETStar connectors

Pin /Socket	Function
1	Power ground
2	RS-232 (RX)
3	Analog ground
4	V in
5	RS-232 (TX)
6	Analog output

Input power of 7–15 VDC is applied to pin 4. The power supply current returns through the common ground pin. Data is sent out the serial output pin (pin 5).

1.2 Test Cable

Some **Digital units** ship with a test cable that allows you to supply power to the WETStar and provides a DB9 serial connector for RS232 output.

1.3 Delivered Items

The standard WETStar delivery includes:

- Tubing nipples that allow a small pump to be connected to the instrument
- small stick of fluorescing plastic material for functionality checks:
  - pink stick for chlorophyll, Rhodamine and phycoerythrin
  - blue stick for CDOM
  - yellow stick for Uranine
- this manual
- instrument-specific characterization sheet
- pigtail lead with mating connector (except some digital units)

2. Theory of Operation

The WETStar miniature fluorometer allows the user to measure relative chlorophyll, CDOM, or other concentrations by directly measuring the amount of fluorescence emission from a given sample of water. The sample media is pumped through a quartz tube mounted through the long axis of the instrument. These samples, when excited by the WETStar internal light source, absorb energy in certain regions of the visible spectrum and emit a portion of this energy as fluorescence at longer wavelengths. Figure 3 shows a simplified illustration of how the WETStar works.

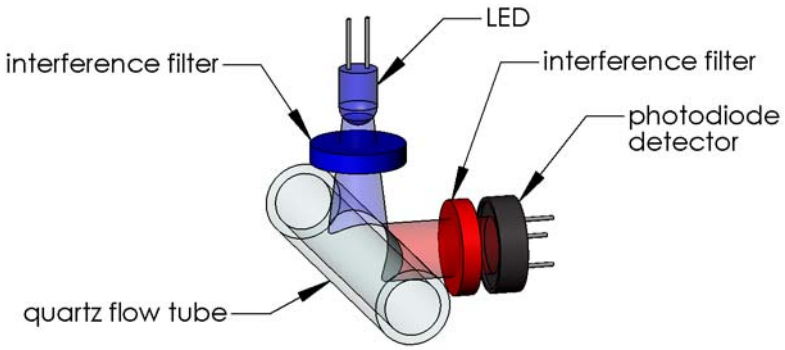

Figure 3. Light path through WETStar

2.1 Chlorophyll WETStar

This WETStar is primarily designed to measure the fluorescence of chlorophyll-containing phytoplankton, which absorb light of wavelengths between 400 and 520 nm and emit light between 670 and 730 nm. The chlorophyll WETStar uses two bright blue LEDs (centered at approximately 470 nm and modulated at 1 kHz) to provide the excitation. Blue interference filters are used to reject the small amount of red light emitted by the LEDs. A detector positioned at 90 degrees to the axis of the LED mounts measures the emitted light from the sample volume. The approximately 0.25 cm^3 sample volume is defined by the intersection of the excitation light with the field of view of the detector, within the quartz flow tube. A red interference filter is used to discriminate against the scattered blue excitation light. The red fluorescence emitted at 90 degrees is synchronously detected at 1 kHz by a silicon photodiode. The amplified and demodulated voltage output of the photodiode is provided to the user for connection to a digital voltmeter, an A/D converter or RS232 input. The instrument contains two LEDs, doubling the excitation light, as well as mirrors and lenses to optimize the instrument's performance.

2.2 CDOM WETStar

The colored dissolved organic matter (CDOM) WETStar is primarily designed to measure the fluorescence of CDOM, which absorbs light in the ultraviolet and emits light in the blue wavelengths. The CDOM WETStar uses two UV LEDs (centered at approximately 370 nm and modulated at 1 kHz) to provide the excitation. A detector, positioned at 90 degrees to the axis of the LED mounts, measures the emitted light from the sample volume. The approximately 0.25 cm^3 sample volume is defined by the intersection of the excitation light with the field of view of

the detector, within the quartz flow tube. A blue interference filter is used to discriminate against the scattered blue UV excitation light. The blue fluorescence is synchronously detected at 1 kHz by a silicon photodiode. The amplified and demodulated voltage output of the photodiode is provided to the user for connection to a digital voltmeter, an A/D converter or RS232 input. The instrument contains two LEDs, doubling the excitation light, as well as mirrors and lenses to optimize the instrument's performance.

2.3 Uranine WETStar

The uranine (fluorescein) WETStar uses two bright blue LEDs (centered at approximately 470 nm and modulated at 1 kHz) to provide the excitation. Blue-green interference filters are used to reject the small amount of red light emitted by the LEDs and produce the 485 nm excitation light. A detector, positioned at 90 degrees to the axis of the LED mounts, measures the emitted light from the sample volume. The approximately 0.25-cm^3 sample volume is defined by the intersection of the excitation light with the field of view of the detector, within the quartz flow tube. A green interference filter is used to discriminate against the scattered blue excitation light. The green fluorescence emitted at 90 degrees is synchronously detected at 1 kHz by a silicon photodiode. The amplified and demodulated voltage output of the photodiode is provided to the user for connection to a digital voltmeter or an A/D converter. The instrument contains two LEDs, doubling the excitation light, as well as mirrors and lenses to optimize the instrument's performance.

2.4 Rhodamine WETStar

This WETStar is primarily designed to measure the fluorescence of rhodamineWT dye, which absorbs light of wavelengths between 470 and 550 nm and emits light around 590 nm. The rhodamine WETStar uses two bright blue LEDs (centered at approximately 470 nm and modulated at 1 kHz) to provide the excitation. Blue interference filters are used to reject the small amount of red light emitted by the LEDs. A detector, positioned at 90 degrees to the axis of the LED mounts, measures the emitted light from the sample volume. The approximately 0.25 cm^3 sample volume is defined by the intersection of the excitation light with the field of view of the detector, within the quartz flow tube. A orange interference filter is used to discriminate against the scattered blue excitation light. The orange fluorescence emitted at 90 degrees is synchronously detected at 1 kHz by a silicon photodiode. The amplified and demodulated voltage output of the photodiode is provided to the user for connection to a digital voltmeter, an A/D converter or RS232 input. The instrument contains two LEDs, doubling the excitation light, as well as mirrors and lenses to optimize the instrument's performance.

2.5 Phycoerythrin WETStar

This WETStar is primarily designed to measure the fluorescence due to the presence of phycoerythrin pigment in cyanobacteria, with excitation and emission wavelengths of approximately 520 and 570 nm, respectively. The phycoerythrin WETStar uses two green LEDs (centered at approximately 520 nm and modulated at 1 kHz) to provide the excitation. Interference filters are used to filter out-of-band light emitted by the LEDs. A detector, positioned at 90 degrees to the axis of the LED mounts, measures the emitted light from the sample volume. The approximately 0.25 cm^3 sample volume is defined by the intersection of the

excitation light with the field of view of the detector, within the quartz flow tube. The fluorescence emitted at 90 degrees is synchronously detected at 1 kHz by a silicon photodiode. The amplified and demodulated voltage output of the photodiode is provided to the user for connection to a digital voltmeter, an A/D converter or RS232 input. The instrument contains two LEDs, doubling the excitation light, as well as mirrors and lenses to optimize the instrument's performance.

3. Instrument Operation

WETStar meters are available with either analog or digital output. Both outputs are proportional to the amount of fluoresced light emitted. This value is, in turn, proportional to the phycocyanin concentration in the sample volume. Analog output ranges from 0–5 V, and the digital output ranges from 0–4095 counts.

A four-pin bulkhead connector and matching pigtail provide the power, ground, analog out and analog return or digital signal(s). The pinouts are clearly described in section 1.2. The analog out and/or digital signals can connect to a data acquisition system of your choice. WETStar is designed to connect directly to many CTD systems and is compatible with other platforms that can provide power and accept a 0–5 VDC analog signal. The digital signal is output to a PC or data logger using the test cable provided.

3.1 Connector Check

Push the pigtail straight on to the connector sockets without wiggling the pigtail from side to side. The connection is very snug, and it helps to apply a thin coat of silicon grease to the connector sockets. Give an additional push to remove any trapped air and "seat" the pigtail. Screw the lock collar securely to the bulkhead connector. To remove the pigtail, unscrew the lock collar then grasp the body of the pigtail (not the wire) and pull straight out. Many connectors are damaged by rocking the pigtail connector from side to side as they are pulled out.

3.2 Electrical Checkout

WARNING!

Do not deliver more than 15 VDC to the WETStar. The WETStar is protected against high transient voltage events with a Transient Suppression Device. When voltage transients higher than 15 VDC occur the transient voltage suppression device turns on and shunts this voltage to ground. If an input power higher than 15 VDC is applied to the WETStar, this device will turn on. If left in this condition the transient voltage suppression device will fail and damage to the WETStar may occur.

Electrical checkout of WETStar is straightforward. Apply 7–15 VDC to the instrument to provide power to the LEDs and electronics. Ensure that positive voltage is applied to V+ (socket 3), and common or ground is applied to the ground (socket 1) (the large hole in the bulkhead connector). A common 9-volt battery makes an ideal power supply for bench testing. With the proper voltage applied to sockets the ground (socket 1) and V+ (socket 3), the LEDs should illuminate the quartz flow tube. This light can be seen when looking straight into the flow tube with the plastic fluorescence test stick inserted.

3.3 Analog Signal Output Check

Connect analog out (socket 2) and analog return (socket 4) to a digital multimeter. With the flow tube clean and dry, the analog output voltage should read approximately 0.05–0.5 VDC. Insertion of the fluorescent plastic test stick into the flow tube should produce a signal level at or near saturation (~5 VDC).

3.4 Digital Signal Output Check

The RS-232 output from the digital WETStar is a single column of numbers whose values range between 0 and 4095 counts. Connect the test cable to the WETStar. Connect the DB-9 connector to a PC running a terminal communications program such as HyperTerminal. Set the data rate to 9600 baud, 8 data bits, 1 stop bit, no parity. Connect a 9 V battery to the test cable. Output should read approximately 40–500 counts. Inserting the plastic fluorescent stick should increase the signal to near saturation, or 4095 counts.
Note that RS-232 protocol is limited to a nominal cable length of 5 meters (15 ft). WET Labs' experience is that these cables can be significantly longer, but they should be tested before deployment.

3.5 Deployment

WETStar can be deployed in either a non-pumped flow through mode or a pumped configuration. We highly recommend using a pump because, as one would expect from considerations of phytoplankton physiology, there is a flow rate dependence of the signal. A pump, used during calibration and during field work, will provide a consistent flow and ensure the highest quality data. We supply threaded tubing nipples for the inlet and outlet flow tube ports to aid in plumbing a pump and/or water traps. If you deploy WETStar in a flow through mode, best results will be obtained by lowering the instrument steadily at 0.2 to 1.0 meters per second. This is compatible with the descent rate requirements of many small CTDs.

If the instrument is used in a free flow mode, it is important to ensure that the flow tube inlet/outlets are "seeing" a clear water path during descent. Since WETStar's size makes it easy to tuck away inside a cage, this can present a problem. One solution would be to add Tygon tubing to the fittings on the flow tube that are in turn connected to water traps (funnel-type devices which are mounted with their wide end facing in the direction of deployment).

If you use a small pump to flush the flow cell, the recommended flow rate is in the range of 10 to 30 ml/sec. Laboratory tests have shown that, for phytoplankton cultures, increasing the flow rate up to 30 ml/sec decreases the signal but slightly improves the signal to noise ratio. The flow rate which provides the best signal to noise ratio is 25 ml/sec. A good pump for this purpose is Sea-Bird Electronics' SBE-05T, which is a small, low powered pump which has an adjustable motor speed so that flow rate can be precisely controlled. Flow rate dependence for chlorophyll WETStars is discussed further in section 5.

Note that while CDOM does not display the physiological response behavior of phytoplankton, flow rates that are very low or very high may prove problematic: UV bleaching can occur at very low rates, and the instrument's response may be limiting at very high rates.

3.6 Data Collection

Analog WETStars must be connected to a host system that will receive the analog voltage output and digitize it. Many oceanographic instruments such as CTDs, radiometers, and data loggers are equipped with analog input channels and carry on-board A/D converters.

Adding the instrument to a CTD or other host solves several other problems. Since the data is merged with the CTD data, correlating the WETStar output with depth or time is done automatically. If one is building a logger or interface, it will be necessary to provide some pressure or time reference to stamp the fluorescence data, tying it to the rest of the physical data.

Analog WETStar output is limited to a current of 10 mA or less. Its output impedance is approximately 500 ohms that effectively limits the drive current. Therefore, the electrical signal will degrade over a long electrical wire due to the electrical resistance of the cable. For best results, the analog signal should be fed directly into an A/D converter and the digital signal should be sent up the cable. One such option is to use WET Lab's DH-4 data logger, a sub-surface data logging system that can handle up to three analog signals simultaneously, as well as two digital signals if necessary.

3.7 Data Analysis

Because of the varied environments in which each user will work, it is important to perform characterizations using similar seawater as you expect to encounter *in situ.*

3.8 Upkeep and Maintenance

WETStar is a very compact instrument and its maintenance can be easily overlooked. However, the miniature fluorometer is a precision instrument and does require a minimum of routine upkeep. After each cast or exposure of the instrument to natural water, flush the instrument with clean fresh water, paying careful attention to the flow tube. Soapy water will cut any grease or oil accumulation. The tube is high quality quartz that can easily be broken or scratched so use caution. Do not use a dowel or stiff brush in the tube. A long cotton swab works nicely for cleaning the tube. At the end of an experiment, the instrument should be rinsed thoroughly, air-dried and stored in a cool, dry place. Solvents such as methanol may also be used to clean the tube.

4. Characterization

The chlorophyll WETStar is typically configured for one of two measurement ranges: 0.03–75.0 µg/l or 0.06–150 µg/l. This is done at WET Labs using a fluorescent stick and adjusting the electronic gain of the WETStar for a corresponding specific output value.
As is the case with other fluorometers, detailed characterization must be performed by the user to determine the actual zero point and scale factor for his/her particular use.

4.1 Chlorophyll Characterization

To measure the WETStar output voltages for tuning and characterization, the analog fluorometer is connected to a 16-bit analog-to-digital (A/D) converter. The A/D outputs the voltages in a standard RS-232 serial text format that is collected with a terminal program. A spreadsheet is then used to perform calculations on the collected values.

A Scale Factor is used to convert the fluorescence response of the instrument into chlorophyll-a concentration. The Scale Factor is determined at WET Labs during a cross calibration using a solid fluorescent standard and a reference fluorometer whose chlorophyll fluorescence response has been characterized in a laboratory using a mono-species lab culture of *Thalassiosira weissflogii* phytoplankton.

Refer to Appendix B for details on performing a laboratory or field calibration on the WETStar.

The WETStar sensitivity is adjusted to be within certain limits when a controlled fluorescence standard is introduced into the sample volume. The standard is nominally equivalent to 50 µg/l of chlorophyll. On the WS3S models, the output voltage is adjusted for 3.0 ± 0.150 V), and on the WS1S, the output is 1.5 ± 0.150 V).

4.2 CDOM Characterization

The current substance used to characterize the CDOM WETStar is a quinine sulfate dihydrate (QSD) solution at a concentration of 100 parts per billion (ppb).

WARNING
If you are not experienced or trained in the safe and proper techniques of working with chemicals and acids, seek proper assistance.

The gain of the instrument is adjusted based on the signal level generated from the 100 pbb QSD solution. Linearity of the instrument is checked using a dilution series of QSD. The sensitivity of individual instruments may vary due to specific requirements of deployment conditions. Refer to the Characterization Sheet that accompanied your instrument for precise values regarding range and sensitivity to QSD. It is important to note that QSD is used in the characterization of the CDOM WETStar and does not constitute a calibration of the instrument's response to naturally occurring CDOM.

The CDOM WETStar range and sensitivity is adjusted to be within certain limits when a QSD solution of a certain concentration is introduced into the sample volume. The CDOM WETStar output is adjusted by setting the gain at several operational amplifier stages. Gain is set with fixed-value precision resistors.

The variable nature of the fluorescence due to naturally occurring CDOM makes it impossible for an accurate calibration to be done in a lab for all situations. The CDOM WETStar, therefore, is not calibrated to CDOM, but rather QSD, which has historically been used to establish the response and sensitivity of fluorometers used in CDOM fluorescence applications. The absorption and emission characteristics of QSD allow it to be used as a proxy for CDOM.

An instrument-specific Clean Water Offset (CWO) and 100 ppb QSD measurement standard are provided on the characterization sheet that shipped with your WETStar. The conversion of raw counts to QSD equivalent concentration is straightforward using the equation:

$$[X]_{sample} = (C_{sample} - C_{cwo}) * \text{Scale Factor}$$

where:

$[X]_{sample}$ = concentration, sample of interest
C_{sample} = raw counts output when measuring a sample of interest (counts)
C_{cwo} = Clean Water Offset (counts)
Scale Factor (SF): CDOM = multiplier in ppb QSD-counts
$[X]_{standard}$ = concentration, known QSD equivalent sample

Then, $SF = [X]_{standard} \div (C_{sample} - C_{cwo})$

For example, if the CWO reading was 60 counts and a known concentration of 65 ppb provided a signal of 3500 counts, your scale factor would be:

65.0 µg/l ÷ (3500 - 60) counts = 0.018 ppb-counts.

4.3 Uranine/Rhodamine/Phycoerythrin WETStar Characterization

The characterization procedure is to prepare a dilution series of the dye solution. Uranine, (or fluorescein) and RhodamineWT dye (also used for the phycoerythrin meter) are available from chemical supply dealers. The sensitivity of the WETStar is adjusted such that the slope of the linear regression and range of the dilution series is appropriate to the range requested by the user.

4.4 Gain Adjustment

The output is adjusted by setting the gain in several operational amplifier stages in the WETStar. Gain, or sensitivity, is set with fixed-value resistors.

4.4.1 Pure Water Blank

Pure, de-ionized water is used to set the "zero" voltage of the WETStar. This zero voltage is set for approximately 0.070 ± 0.030 V) on most WETStar models. The WETStar employs an offset voltage circuit. Water blank is adjusted with two fixed voltage divider resistors at the factory.

4.4.2 Response Time (Time Constant)

The specified time constant for the analog WETStar is 0.167 seconds; 0.125 for the digital. This time constant is the RC value, computed by 1/RC.

To verify the time constant, the step response is observed on an oscilloscope. A sample is introduced that produces a full-scale reading. The sample is then quickly removed, and the decay is observed on the oscilloscope. The output voltage must reach a value of 66 percent of the original within 0.167 (or 0.125 for digital) seconds. A nominally full-scale output is obtained after six time constants.

4.5 Final Testing

4.5.1 Pressure

To ensure the integrity of the housing and seals, the WETStar is subjected to a wet hyperbaric test before final testing. The testing chamber applies a water pressure of at least 50 PSI. The rated depth of the WETStar is 600 meters.

4.5.2 Mechanical Stability

Before final testing, the WETStar is subjected to a mechanical stability test. This involves subjecting the unit to mild vibration and shock. The air, water, and sample voltages must remain the same before and after the mechanical stability test.

4.5.3 Temperature Stability

To verify temperature stability, the WETStar is immersed in an ice bath. The starting temperature is typically 23–30 degrees Celsius, and the ending temperature is 1–5 degrees Celsius. A voltage sample is collected every 30 seconds, with a 0.5 second smoothing. Specifications assert that the maximum variation per degree Celsius is 1.25 mV (2 counts).

4.5.4 Electronic Stability

This value is computed by collecting a sample once per minute for twelve hours or more. The smoothing time for this one sample is 0.5 seconds. After the data is collected, the minimum and maximum values are determined, and the difference between these two is divided by the number of hours the test has run. The result is the stability value listed on the characterization sheet. The stability value must be less than 2.0 mV (3 counts)/hour.

4.5.5 Full Scale Verification

The specified maximum output of the WETStar is nominally five volts, or for digital meters, 4095 counts. The full-scale voltage is reported in the characterization sheet.

4.5.6 Noise

The noise value is computed from a standard deviation over 60 samples. These samples are collected at one-second intervals for one minute. The smoothing (averaging) time for these samples is 0.5 seconds. A standard deviation is then performed on the 60 samples, and the result is the published noise on the characterization sheet. The calculated noise must be below 1.5 mV (3 counts).

4.5.7 Final Water Blank Test

De-ionized, pure water is introduced into the sample volume. The output voltage must be 0.070 ± 0.030 V for most WETStar types. Value is recorded on the characterization sheet.

4.5.8 Final Standard Test

A standard sample specific to the measurement range of the WETStar being tested is placed in the sample volume. The output reading is recorded on the instrument-specific Characterization Sheet.

4.5.9 Voltage and Current Range Verification

To verify that the WETStar operates over the entire specified voltage range (7–15 V), a voltage-sweep test is performed. The WETStar is operated over the entire voltage range, and the current and operation is observed. The total power consumption (voltage times current) must remain below 500 mW (900 mW for digital) over the entire voltage range.

4.5.10 Linearity

Linearity tests are performed on many WETStars. This linearity test consists of a complete Coproporphyrin dilution series for chlorophyll meters, a QSD series for CDOM meters, and a uranine series for uranine meters. The linear regression “R-squared” value must be better than 0.9900.

Appendix A. Chlorophyll WETStar Flow Rate Dependence

Fluorescent signals from phytoplankton samples exhibit some dependence on the flow rate of the sample water through the measurement chamber. Because of WETStar's unique size and flow tube technology, providing for a uniform flow rate is highly desirable. For this reason, we recommend using a small submersible pump with a known flow rate. Free-flow (un-pumped) measurements are possible, but care must be taken to use a steady profiling rate on the order of one meter per second to provide the proper flushing of the flow tube. It is important to note that when profiling in free-flow mode, it is possible for the descent of the profiling package to come to a stop or even reverse direction briefly due to sea-state and ship motion. Figure 4 shows how the voltage output of the WETStar can vary with flow rates ranging from about 3 to 30 ml/sec. A small, constant volume pump was placed in line with a closed loop flow system fitted with a throttle valve to control the flow rate. It should be noted that the relative response only varies by 15–18 percent over the entire range so that useful data can be obtained even in situations where the flow rate is unknown.

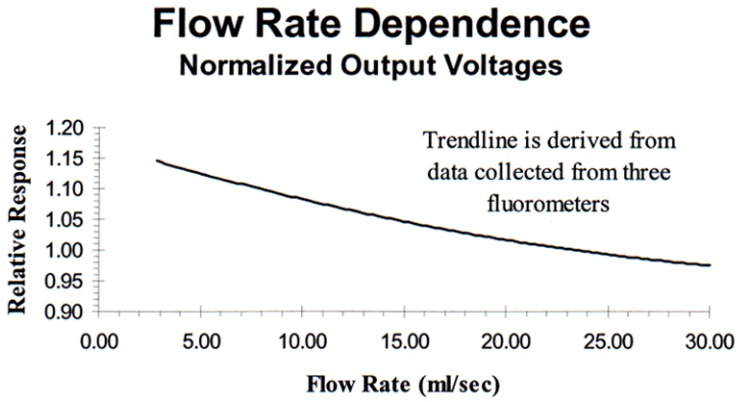

Figure 4. Flow rate dependence for *Thalassiosira weissflogii*.

Output voltages are normalized to the voltage value recorded at 24 ml/sec. Using the same flow rate in the field that was used during one's calibration will ensure consistent results.

Appendix B. Calibration Details for Chlorophyll WETStar

This section is provided by Richard Davis, Oregon State University.

WETStar is shipped pre-configured to provide accurate, linear response over one of two dynamic ranges: 0.03–75.0 or 0.06–150 µg/l. However, because of myriad calibration techniques and different properties of the natural waters from which "blanks" will be prepared, it is important that an experiment-specific calibration is done before (and after) each major cruise or event. The key to obtaining high quality data is to determine the instrument's response to the conditions that will be found in the field. Because of the many different applications involving fluorometric chlorophyll determinations, detailed calibration of the instrument must be done by the user.

Introduction and Caveats

The purpose of calibrating an *in situ* fluorometer is to be able to convert its in-water signal to an absolute value of chlorophyll-*a*. In theory this should be a simple process of measuring the voltages from the instrument obtained from a dilution (or addition) series of a phytoplankton culture of known concentration, creating a linear regression of the recorded voltages against chlorophyll concentration and then obtaining a calibration coefficient. Problems arise from the fact that the optical properties of phytoplankton are functions of size, shape, pigmentation, taxonomic composition, photo-adaptation and physiological status. For example, exposure to supersaturating light will cause an immediate (time scale of seconds) depression in fluorescence with any change in chlorophyll concentration happening very much slower (time scale of hours) (Kiefer 1973, Cullen et al.1988). However, since it is unreasonable to calibrate a fluorometer with every species of phytoplankton at all different physiological states, one has to simply be aware of the problems and go forth. Thus any conversions of *in situ* fluorometry into chlorophyll-*a* concentration are estimates at best and guesses at worst. Over the time scale of a mooring deployment a fluorometer will probably estimate chlorophyll-*a* within a factor of 2 (Lorenzen 1966).

Calibration Protocol

The following procedures involve using some improvisational techniques and equipment. For example, the Sea-Bird SBE-5 pump is designed for submersible work and may prove difficult or impossible for some people to use in the way described. This calibration procedure also assumes the person performing calibration is already familiar with the spectrophotometric extraction method for determining chlorophyll concentration.

Calibration should be a two-phase process. The first phase, a serial addition procedure, occurs in the lab, the second, a simple correlation procedure, in the field.

Lab Calibration

Materials needed:

- WETStar fluorometer
- SBE pump
- cables for fluorometer and pump
- tubing for fluorometer
  - inlet
  - outlet
- thermometer
- scintillation vials w/10 ml 90% acetone
- set of volumetric pipettes
  - 5 ml
  - 10 ml
  - 25 ml
- pipette bulb
- large tub for immersing instruments
- lab notebook
- DC power supply
- digital voltmeter
- ring stand w/2 large clamps
- 50, 100 and 500 ml graduated cylinders
- phytoplankton culture
- stopwatch
- some 500 ml beakers
- GF/F filters
- forceps
- filter rig w/pump, tubing, traps

It is critical that the entire calibration process be performed under non-varying conditions. Changes in temperature or light will affect the *in vivo* fluorescence of the phytoplankton culture and possibly the instrument. It is recommended that the room be dimly lit and that the culture be allowed to sit in the calibration area for 30 minutes prior to use. The instrument should be equilibrated to calibration temperature for at least 4 hours.

Obtain a culture of late logarithmic phase phytoplankton and some of the culture media. Use a species of phytoplankton (or at least genera) that you are likely to encounter in the field. If time, energy and materials allow, use two species of different groups (e.g., a diatom and a chrysophyte) for comparison purposes. Absolute concentration of chlorophyll-*a* in the culture should be no more than 50 µg/l (you can start to see color by eye at about 20 µg/l, at which point the culture is starting to slow down and enter the stationary phase). Filter approximately 50–100 ml onto a GF/F filter for spectrophotometric chlorophyll determination. You should be able to easily see color on the filter. If you can't, filter more. Place the filter into 10 ml of 90 percent acetone and store in a freezer for 24 hrs. Record the optical density of the solution at 750, 664, 647, and 630 nm. Calculate pigment concentrations by:

$$C_a = 11.85*OD_{664} - 1.54*OD_{647} - 0.08*OD_{630}$$
$$C_b = 21.03*OD_{647} - 5.43*OD_{664} - 2.66*OD_{630}$$
$$C_c = 24.52*OD_{630} - 1.67*OD_{664} - 7.60*OD_{647}$$

where a, b, and c denote chlorophyll-*a*, -*b* and -*c*, respectively, and all optical densities have had the 750 nm signal subtracted.

Set up the WETStar fluorometer and pump on a ring stand according to Figure 5. Provide power to the fluorometer and pump via a DC power supply. Record all voltages from the fluorometer with a voltmeter. Power up the fluorometer and let it warm up for 10 minutes. At the end of this period record the voltage as the air blank. Begin pumping a known volume of culture medium through the fluorometer. Check for and clear bubbles. Allow the fluorometer to stabilize and record the voltage as the seawater blank. Add an aliquot of culture to the blank culture medium with a volumetric pipette. The aliquot should be enough that you would expect to see an increase in the fluorometer voltage between 0.5 and 1 volt. In the scenario of the WETStar set up for the lower range (0–75 µg/l), a culture containing approximately 50 µg/l chlorophyll, and a blank culture medium volume of 400 ml the aliquot would be approximately 25 ml. After the reading has stabilized record the voltage and volume added. Continue with the serial additions until you saturate the fluorometer. Clean the fluorometer after calibrating.

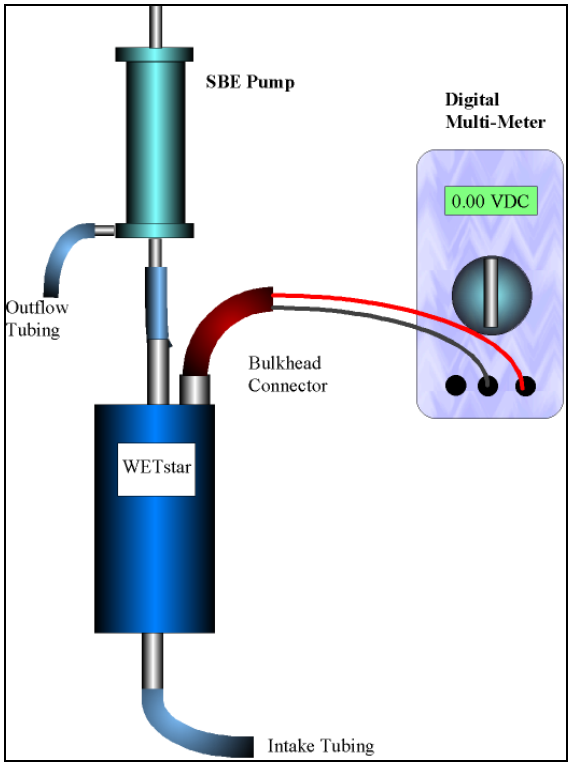

Figure 5. Instrument setup

Field Calibration

There are two goals to the field calibration. The first is to verify that the lab calibration is valid in the field. The second is to detect any changes in the fluorometer over time. To achieve these goals chlorophyll-*a* samples should be taken as often and as close as possible to the mooring. Record the time that the Niskin bottle containing the sample was tripped. Preferably samples would be taken throughout a 24 hr period to investigate the effects of irradiance on *in situ* fluorescence.

Appendix C. References

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Marra, J. and C. Langdon. 1993. An evaluation of an *in situ* fluorometer for the estimation of chlorophyll-*a*. Tech. Rep. LDEO-93-1, Lamont-Doherty Earth Observatory.

WETStar

User's Guide

Revision History

Revision	Date	Revision Description	Originator
A	10/07/99	Begin revision tracking	H. Van Zee
B	01/03/00	Change Specifications, Delete section 3.3 (DCR 6)	C. Moore
C	01/06/00	Update document (DCR 8)	D. Hankins
D	03/29/00	Reorder chapters for consistency (DCR 19)	H. Van Zee
E	12/12/00	Update document and illustrations (DCR 75)	D. Hankins, H. Van Zee
F	03/12/01	Correct Copro standard preparation (DCR 90)	D. Hankins
G	11/26/01	Revise references to excitation wavelengths (DCR 164)	J. Kitchen
H	01/23/02	Correct terminology in section 2 and update Figure 1 (DCR 188)	H. Van Zee
I	04/09/02	Add digital WETStar capabilities (DCR 213)	S. Campos. H. Van Zee
J	10/25/02	Delete reference to Schott glass (DCR 246)	S. Campos
K	05/12/03	Add four-pin digital connector diagram and functions (DCR 300)	M. Everett
L	1/11/05	Add phycoerythrin, combine chlorophyll, CDOM, uranine, Rhodamine into single user's guide. (DCR 435)	M. Everett
M	1/13/06	Clarify warranty statement (DCR 481)	A. Gellatly, S. Proctor